Research Article

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Biting deterrence and insecticidal activity of hydrazide-hydrazones and their corresponding 3-acetyl-2,5-disubstituted-2,3-dihydro-1,3,4-oxadiazoles against *Aedes aegypti*

Nurhayat Tabanca,[a*] Abbas Ali,[a] Ulrich R Bernier,[b] Ikhlas A Khan,[a,c,d] Bedia Kocyigit-Kaymakcioglu,[e] Emine E Oruç-Emre,[f] Seda Unsalan[e] and Sevim Rollas[e]

Abstract

BACKGROUND: Taking into account the improvement in insecticidal activity by the inclusion of fluorine in the hydrazone moiety, the authors synthesized new 4-fluorobenzoic acid hydrazides and 3-acetyl-2,5-disubstituted-2,3-dihydro-1,3,4-oxadiazoles, substituting a phenyl group or a heteroaryl ring carrying one or two atoms of F, Cl and Br, and investigated their biting deterrent and larvicidal activities against *Aedes aegypti* for the first time.

RESULTS: The compound 3-acetyl-5-(4-fluorophenyl)-2-[4-(dimethylamino)phenyl]-2,3-dihydro-1,3,4-oxadiazole (17) produced the highest biting deterrent activity (BDI = 1.025) against *Ae. Aegypti*, followed by 4-fluorobenzoic acid [(phenyl)methylene] hydrazide (1). These activity results were similar to those of *N,N*-diethyl-*meta*-toluamide (DEET), which showed a proportion not biting of 0.8–0.92. When compounds 1 and 17 were tested on cloth worn on human volunteers, compound 1 was not repellent for some volunteers until present in excess of 500 nmol cm^{-2}, while compound 17 was not repellent at the highest concentration tested (1685 nmol cm^{-2}). In the larvicidal screening bioassays, only compounds 10, 11, 12 and 17 showed 100% mortality at the highest screening dose of 100 ppm against Ae. aegypti larvae. Compounds 11 and 12 with LD_{50} values of 24.1 and 30.9 ppm showed significantly higher mortality than 10 (80.3 ppm) and 17 (58.7 ppm) at 24-h post-treatment.

CONCLUSION: The insecticidal and biting deterrent activities were correlated with the presence of a halogen atom on the phenyl or heteroaryl substituent of the hydrazone moiety.

Keywords: hydrazide-hydrazones; 1,3,4-oxadiazoles; biting deterrent; repellent; larvicidal; *Aedes aegypti*

1 INTRODUCTION

Mosquito-borne diseases are major health problems worldwide. The yellow fever mosquito, *Aedes aegypti* L., can be found throughout the world and in many areas of high human population. This species transmits arboviruses to human beings, infecting more than 2.5 billion people, mainly in tropical countries.[1] There are no effective vaccines or treatments for disease prevention, and personal protection is necessary to prevent mosquito bites.[2] A synthetic compound, *N*, *N*-diethyl-*meta*-toluamide (DEET), was introduced into the market in the 1950s as a repellent against biting insects and later on new insecticides including pyrethroids have been widely used to control a wide range of insect pests in agriculture and public health situations including mosquito larvae.[2,3] Owing to the continuous use of these insecticides, mosquitoes have developed resistance against them, and hence there is a need to search for new insecticides for controlling these resistant mosquitoes.

* *Correspondence to: Nurhayat Tabanca, National Center for Natural Products Research, The University of Mississippi, University, MS 38677, USA. E-mail: ntabanca@olemiss.edu*

a *National Center for Natural Products Research, The University of Mississippi, University, MS, USA*

b *USDA, ARS, Center for Medical, Agricultural and Veterinary Entomology, Gainesville, FL, USA*

c *Department of Pharmacognosy, School of Pharmacy, The University of Mississippi, University, MS, USA*

d *Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia*

e *Marmara University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Istanbul, Turkey*

f *Gaziantep University, Faculty of Arts and Sciences, Department of Chemistry, Gaziantep, Turkey*

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14. ABSTRACT

BACKGROUND:Taking into account the improvementininsecticidal activityby the inclusionof fluorine inthe hydrazone moiety the authors synthesized new 4-fluorobenzoic acid hydrazides and 3-acetyl-2,5-disubstituted-2,3-dihydro-1,3,4-oxadiazoles substituting a phenyl group or a heteroaryl ring carrying one or two atoms of F, Cl and Br, and investigated their biting deterrent and larvicidal activities against Aedes aegypti for the first time. RESULTS:Thecompound3-acetyl-5-(4-fluorophenyl)-2-[4-(dimethylamino)phenyl]-2,3-dihydro-1,3,4-oxadiazole (17)produced the highest biting deterrent activity (BDI = 1.025) against Ae. Aegypti, followed by 4-fluorobenzoic acid [(phenyl)methylene] hydrazide (1). These activity results were similar to those of N,N-diethyl-meta-toluamide (DEET), which showed a proportion not biting of 0.8?0.92. When compounds 1 and 17 were tested on cloth worn on human volunteers, compound 1 was not repellent for some volunteers until present in excess of 500 nmol cm−2, while compound 17 was not repellent at the highest concentration tested (1685 nmol cm−2). In the larvicidal screening bioassays, only compounds 10, 11, 12 and 17 showed 100% mortality at the highest screening dose of 100 ppm against Ae. aegypti larvae. Compounds 11 and 12 with LD50 values of 24.1 and 30.9 ppm showed significantly higher mortality than 10 (80.3 ppm) and 17 (58.7 ppm) at 24-h post-treatment. CONCLUSION: The insecticidal and biting deterrent activities were correlated with the presence of a halogen atom on the phenyl or heteroaryl substituent of the hydrazone moiety.

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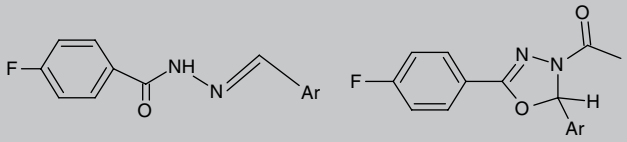
Table 1. Chemical structure of compounds **1** to **20**



1–10 **11–20**

No.	Name	Ar
1	4-Fluorobenzoic acid [(phenyl)methylene]hydrazide	C_6H_5 –
2	4-Fluorobenzoic acid [(4-bromophenyl)methylene]hydrazide	4-BrC_6H_4 –
3	4-Fluorobenzoic acid [(4-fluorophenyl)methylene]hydrazide	4-F-C_6H_4 –
4	4-Fluorobenzoic acid [(4-hydroxyphenyl)methylene]hydrazide	4-OH-C_6H_4 –
5	4-Fluorobenzoic acid [(4-methoxyphenyl)methylene]hydrazide	2-OCH_3-C_6H_4 –
6	4-Fluorobenzoic acid [(4-hydroxy-3-methoxyphenyl)methylene]hydrazide	3-OH-4-OCH_3-C_6H_3 –
7	4-Fluorobenzoic acid [(4-dimethylaminophenyl)methylene]hydrazide	$(CH_3)_2$N-C_6H_4 –
8	4-Fluorobenzoic acid [(4-dimethylaminocinnamyl)methylene]hydrazide	$(CH_3)_2$N-C_6H_4CH=CH-
9	4-Fluorobenzoic acid [(5-bromothiophen-2-yl)methylene]hydrazide	5-Bromo-thiophen-2-yl-
10	4-Fluorobenzoic acid [(2-furanyl)methylene]hydrazide	Furan-2-yl
11	3-Acetyl-5-(4-fluorophenyl)-2-phenyl-2,3-dihydro-1,3,4-oxadiazole	C_6H_5 –
12	3-Acetyl-5-(4-fluorophenyl)-2-(4-bromophenyl)-2,3-dihydro-1,3,4-oxadiazole	4-BrC_6H_4 –
13	3-Acetyl-2,5-bis(4-fluorophenyl)-2,3-dihydro-1,3,4-oxadiazole	4-F-C_6H_4 –
14	3-Acetyl-5-(4-fluorophenyl)-2-(4-hydroxyphenyl)-2,3-dihydro-1,3,4-oxadiazole	4-OH-C_6H_4 –
15	3-Acetyl-5-(4-fluorophenyl)-2-(4-methoxyphenyl)-2,3-dihydro-1,3,4-oxadiazole	2-OCH_3-C_6H_4 –
16	3-Acetyl-5-(4-fluorophenyl)-2-(3-hydroxy-4-methoxyphenyl)-2,3-dihydro-1,3,4-oxadiazole	3-OH-4-OCH_3-C_6H_3 –
17	3-Acetyl-5-(4-fluorophenyl)-2-[4-(dimethylamino)phenyl]-2,3-dihydro-1,3,4-oxadiazole	$(CH_3)_2$N-C_6H_4 –
18	3-Acetyl-5-(4-fluorophenyl)-2-{2-[4-(dimethylamino)phenyl]ethenyl}-2,3-dihydro-1,3,4-oxadiazole	$(CH_3)_2$N-C_6H_4CH=CH-
19	3-Acetyl-2-(5-bromothiophen-2-yl)-5-(4-fluorophenyl)-2,3-dihydro-1,3,4-oxadiazole	5-Bromo-thiophen-2-yl-
20	3-Acetyl-5-(4-fluorophenyl)-2-(furan-2-yl)-2,3-dihydro-1,3,4-oxadiazole	Furan-2-yl

Many hydrazone derivatives have been reported to possess broad-spectrum insecticidal activity and are used as active ingredients for controlling agricultural and horticultural pests.[4–7] For example, the first hydrazone-type insecticide, hydramethylnon, was registered for use in the United States by the Environmental Protection Agency in 1980 to control ants and cockroaches.[7] Benzophenone hydrazone derivatives were reported in 1993 by Syngenta to control *Diabrotica balteata* on corn and in 1996 by Bayer to control *Plutella xylostella* on cabbage.[6] Furthermore, some aryl hydrazono esters[8] and bisacylhydrazine[9] showed significant insecticidal activity against *Aedes albopictus*, *Drosophila melanogaster*, *Aedes aegypti* and *Chironomus tentans*.

Boger *et al.*[4] reported that some benzophenone hydrazone derivatives with a halogen atom and a triflate or perhaloalkoxy group showed good insecticidal activity against *Spodoptera littoralis*. Some heteroaryl hydrazone compounds were introduced into the market by Sankyo Pharmaceuticals Co., Ltd and Nippon Kayaku Co., Ltd in 2000 as acaricides and insecticides.[6] In the present study, various aryl-substituted hydrazones of 4-fluorobenzoic acid hydrazides and 3-acetyl-2,5-disubstituted-2,3-dihydro-1,3,4-oxadiazoles were evaluated for their biting deterrent and larvicidal activity against *Ae. aegypti*.

2 MATERIALS AND METHODS

2.1 Synthesis of test compounds

Hydrazide-hydrazone derivatives **1** to **10** were prepared by the condensation of 4-fluorobenzoic acid hydrazide with appropriate aldehydes. 3-Acetyl-2,5-disubstituted-2,3-dihydro-1,3,4-oxadiazoles **11** to **20** were synthesized by reacting compounds **1** to **10** with acetic anhydride. Physicochemical and spectroscopic characterization of hydrazide-hydrazone compounds **1** to **10** and the corresponding 3-acetyl-2,5-disubstituted-2,3-dihydro-1,3,4-oxadiazoles have been previously described.[10,11] The purity of the synthesized compounds was checked by reversed-phase HPLC [Chromasil C_{18} 3.6 × 150 mm column using acetonitrile and water (50:50 v/v) as the eluent] and elemental analysis. All compounds showed a single and sharp peak with a retention time of 3.219–4.693 min, and also elemental analysis results were within 0.3%. The chemical structures of compounds **1** to **20** are shown in Table 1.

2.2 Biological activity

2.2.1 Insects

The *Ae. aegypti* (L.) mosquitoes used in these studies were from a laboratory colony maintained since 1952, originally from Orlando, Florida, and now at the Mosquito and Fly Research Unit at the Center for Medical, Agricultural and Veterinary Entomology, USDA-ARS, in Gainesville, Florida. This colony is maintained using standard procedures, as described by Pridgeon *et al.*[12] Pupae of *Ae. aegypti* were received from the colony and maintained in the laboratory at 27 ± 2 °C and 60 ± 10% RH as adults emerged. Adults were fed on 10% sucrose solution. Females (5–9 days old) were selected for bioassays. For larval bioassays, the eggs were hatched 1 day prior to testing, and the larvae were maintained at a temperature of 27 ± 2 °C and 70 ± 5% RH in a photoperiod regimen of 12:12 h (L:D).

2.2.2 Mosquito biting bioassays

Experiments were conducted using a six-celled *in vitro* Klun and Debboun (K&D) module bioassay system developed by Klun *et al*.[13] for quantitative evaluation of the bite deterrent properties of candidate compounds for human use. This bioassay method determines specifically measured biting (feeding) deterrent properties of the chemicals. Briefly, the assay system consists of a six-well blood reservoir, with each of the 3 cm × 4 cm wells containing 6 mL of blood. As reported earlier,[14] female mosquitoes feed as well on the CPDA-1 (citrate–phosphate–dextrose–adenine) + ATP as they do on blood. Therefore, CPDA-1 + ATP was used instead of human blood. CPDA-1 was prepared by dissolving 3.33 g of sodium citrate, 0.376 g of citric acid, 4.02 g of dextrose, 0.28 g of monobasic sodium phosphate (Fisher Scientific Chemical Co., Fairlawn, NJ) and 0.346 g of adenine (Sigma-Aldrich, St Louis, MO) in 1026 mL of deionized water. ATP was added to CPDA-1 to yield 10^{-3} M of ATP (AABB 2005). CPDA-1 and ATP preparations were freshly made on the day of the test. *N*,*N*-Diethyl-3-methylbenzamide (DEET) (99.1% purity) was obtained from Sigma Aldrich (St Louis, MO) and used as a positive control. Molecular-biology-grade acetone was obtained from Fisher Scientific Chemical Co. (Fairlawn, NJ). Synthesized hydrazone compounds (**1** to **20**) were tested in this study, and DEET at 25 nmol cm^{-2} was used as the positive control. All the treatments were prepared in acetone. The stock solutions were kept in a refrigerator at 3–4 °C. Treatments were prepared fresh at the time of the bioassay.

The temperature of the solution in the reservoirs was maintained at 37 °C by continuously passing warm water through the reservoir using a circulatory bath. The reservoirs were covered with a layer of collagen membrane. This CPDA-1 + ATP solution membrane unit simulated a human host for mosquito feeding. The test compounds were randomly applied to six 4 cm × 3 cm areas of nylon organdy and positioned over the membrane-covered CPDA-1 + ATP solution with a separator placed between the treated cloth and the six-celled module. A treatment volume of 110 µL was applied per 20 cm^2 area of organdy. A six-cell K&D module containing five females per cell was positioned over cloth treatments covering the six CPDA-1 + ATP solution membrane wells, and trap doors were opened to expose the female mosquitoes to treated cloth. The number of mosquitoes biting through cloth treatments in each cell was recorded after a 3 min exposure, and mosquitoes were prodded back into the cells. These mosquitoes were then squashed to determine the number that had actually engorged the solution. A replicate consisted of six treatments: four test compounds, DEET (a standard bite deterrent compound) and acetone-treated cloth as solvent control. Treatments were randomly assigned to the cells of the module. The 25 nmol cm^{-2} cloth dose of DEET was used as a positive control because it suppresses mosquito biting by 80% in comparison with solvent control.[13] A set of replications was conducted on different days using new batches of insects.

2.2.3 In-cage mosquito repellent assay

Mosquitoes were selected from the stock cages by a hand-draw box.[15] Approximately 500 (± 10%) mosquitoes, consisting primarily of females, were transferred into a test cage (59 000 cm^3 in size with dimensions of 45 cm × 37.5 cm × 35 cm) and allowed to acclimate for 25 ± 2.5 min before repellency assays were initiated.[16] Compounds were weighed and placed in a 2 dram vial to which 2 mL acetone was added. The initial weight of Compounds was measured, so that when one-half (1 mL of solution) removed, and a 50 cm^2 muslin cloth was added to the vial. The 1 mL solution that remained in the vial produced an initial concentration of 1.500 µg cm^{-2} on cloth. Serial dilutions were then made analogously such that the concentrations on the cloth for the remaining 1 mL solution were 750, 375, 187, 94, 47, 23, 11 and 5 µg cm^{-2}. Vials were sealed and stored at −4 °C in a freezer until testing (normally <48 h). A test involved removing cloth from the vial and attaching it by staples onto two sections of card stock (5 cm × 2.5 cm). Pieces of masking tape (2.5–5.0 cm long) secured the cloth onto the card stock. The card and cloth assembly was then placed on a drying rack for 3–5 min before testing.

A single test consisted in covering the hand of a volunteer with a soft-embossed long-cuff poly glove (Atlantis Products, Mankato, MN), followed by a powder-free latex glove (Diamond Grip; Microflex Corporation, Reno, NV). A knee-high stocking (Leggs everyday knee highs; Winston-Salem, NC) was then placed over the gloved hand and arm. A plastic sleeve of polyvinyl was the final layer affixed over the stocking-covered arm. The plastic sleeve was sealed around the arm by a Velcro™ strip. About halfway between the wrist and elbow, a 4 × 8 cm opening in the sleeve allowed assessment of mosquito landing and biting behavior. Attractive odors from the skin surface emanated and attracted mosquitoes to the opening. During testing, this 32 cm^2 open area was covered with compounds treated muslin cloth. The order in which treated cloths were tested was randomized among volunteers. This randomization helps to minimize any effects from day-to-day variation.

A test started when the arm with sleeve and cloth were inserted into the mosquito cage. If 0–4 bites were received during the 1 min, the dosage of repellent on the cloth was considered to have 'passed'. A treatment in which five bites (out of 500 mosquitoes in the cage) occurred in 1 min was considered a failure. Normally, an intermediate dosage (e.g. 187 µg cm^{-2}) was tested first. Depending on whether this concentration passed or failed, higher or lower treatment concentrations were evaluated with all subjects until each had pinpointed their individual concentration that produced the 1% (five-bite) failure point. If the 1500 µg cm^{-2} (or highest) concentration on cloth was not efficacious (more than five bites in 1 min), then the minimum effective dosage (MED) was noted as ineffective at the highest concentration tested. Because mosquitoes become fatigued upon repeated exposure to repellent and attractant odors from the arm, a limit of ten successive tests were conducted, after which the caged mosquitoes were allowed a 15 min recovery period. All units were converted from mg to nmol for prevent contrast.

Three male volunteers (two of whom tested twice) and a female volunteer participated in the studies of MED.[17] During a test, volunteers wore a patch and tested each patch for 1 min intervals. Patches were rotated between the volunteers, and thus no patch was evaluated beyond 10 min after the 3 min drying period to avoid any bias that may result from evaporative loss of the treatment from the cloth during the duration of the test. The subjects provided written informed consent. The protocol was approved by the University of Florida Human Use Institutional Review Board-01 (Study No. 636–2005).

2.2.4 Larval bioassays

Bioassays were conducted using the bioassay system described by Pridgeon *et al*.[18] to determine the larvicidal activity of the synthesized hydrazone-type compounds (**1** to **20**) against *Ae. aegyti*. In brief, the eggs were hatched under vacuum (1 h) by

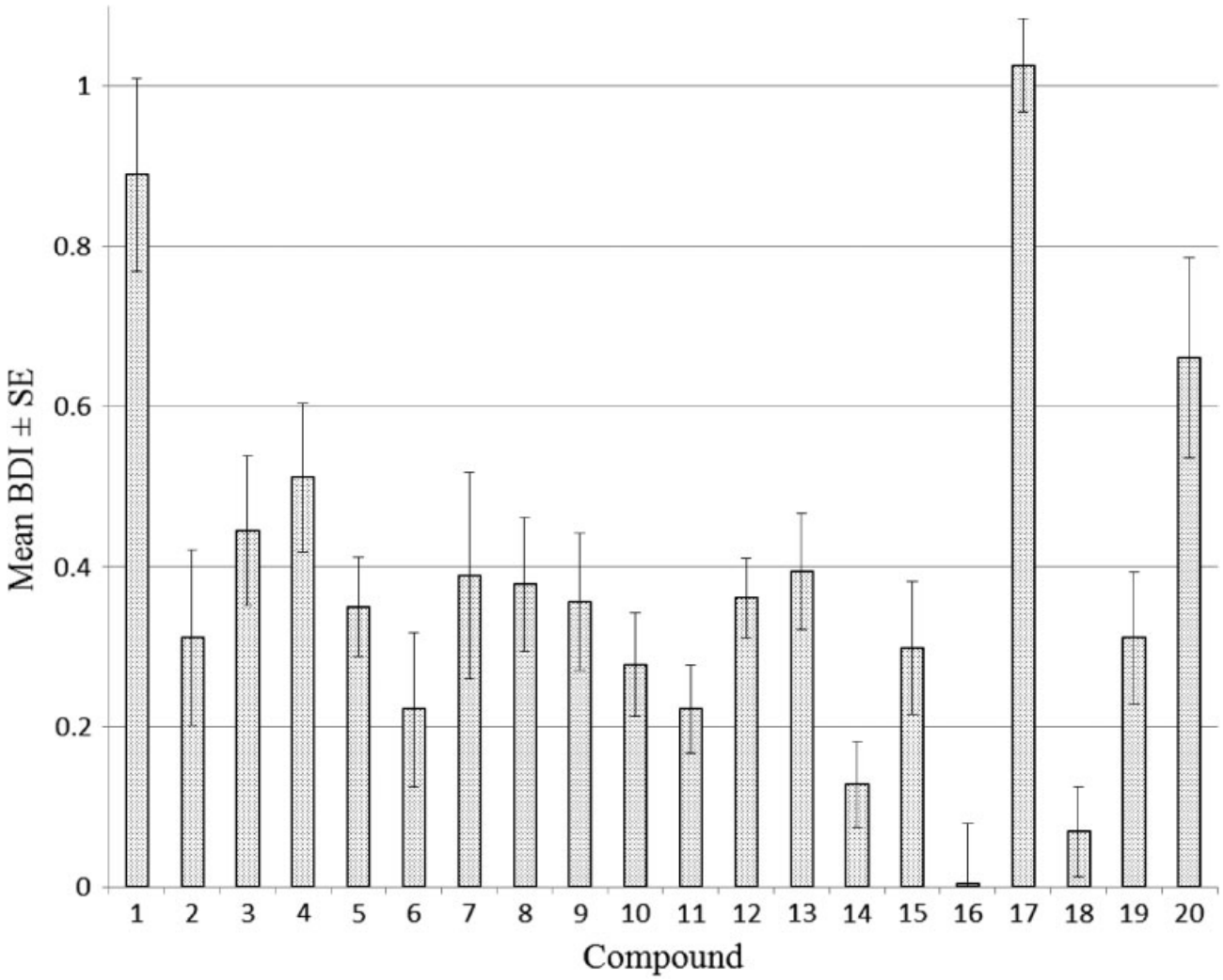


Figure 1. Biting deterrence index (BDI) of hydrazide-hydrazone (**1** to **10**) and 3-acetyl-2,5-disubstituted-2,3-dihydro-1,3,4-oxadiazole (**11** to **20**) compounds against *Aedes aegypti* females. All compounds were tested at a concentration of 25 nmol cm^{-2}. Acetone was the solvent control, and DEET at 25 nmol cm^{-2} was used as positive control. The proportion not biting ranged between 0.8 and 0.92 in DEET, whereas the controls showed values between 0.29 and 0.39.

placing a piece of paper towel with eggs in a cup filled with 100 mL of deionized water containing a small quantity of larval diet. Larvae were removed from vacuum and held overnight in the cup in a temperature-controlled chamber maintained at a temperature of 27 ± 2 °C and 70 ± 5% RH in a photoperiod regimen of 12:12 h (L:D). Five one-day-old first-instar *Ae. aegypti* were added to each well of 24-well plates placed on an illuminated light box by using a disposable 22.5 cm Pasteur pipette with a droplet of water. A quantity of 50 µL of larval diet (2% slurry of 2:1 alfalfa pellets and hog chow) was added to each well by means of a Finnpipette stepper (Thermo Fisher, Vantaa, Finland). All chemicals to be tested were diluted in dimethyl sulfoxide (DMSO). A quantity of 11 µL of the test chemical was added to the labeled wells, and in control treatments 11 µL of DMSO alone was added. Each well had a total volume of 1.1 mL. After the treatment, the plates were swirled in clockwise and counterclockwise motions and front and back and side to side 5 times to ensure even mixing of the chemicals. Larval mortality was recorded at 24, 48 and 72 h post-treatment. Larvae that showed no movement in the well after manual disturbance of water by a pipette tip were recorded as dead. A series of dosages was used in each treatment to obtain a range of mortality. Treatments were replicated 15 times in each compound.

2.2.5 *Statistical analyses*

As the K&D module bioassay system can handle only four treatments along with negative and positive controls, in order to make direct comparisons among more than four test compounds and to compensate for variation in overall response among replicates, repellency was quantified as the biting deterrence index (BDI). BDI values were calculated using the following formula:

$$[\mathrm{BDI}_{i,j,k}] = \left[\frac{\mathrm{PNB}_{i,j,k} - \mathrm{PNB}_{c,j,k}}{\mathrm{PNB}_{d,j,k} - \mathrm{PNB}_{c,j,k}}\right]$$

where $\mathrm{PNB}_{i,j,k}$ denotes the mean proportion of females not biting test compound i for replication j and day k (i = 1–4, j = 1–5, k = 1–2), $\mathrm{PNB}_{c,j,k}$ denotes the mean proportion of females not biting the solvent control for replication j and day k (j = 1–5, k = 1–2) and $\mathrm{PNB}_{d,j,k}$ denotes the mean proportion of females not biting in response to DEET (positive control) for replication j and day k (j = 1–5, k = 1–2). This formula adjusts for variation

in response among replication days and incorporates information from the solvent control as well as the positive control.

A BDI value of 0 indicates an effect similar to that of acetone. A BDI value significantly greater than 0 indicates an antibiting effect relative to that of acetone. BDI values not significantly different from 1 are statistically similar to DEET. BDI values were analyzed using SAS Proc ANOVA (SAS Institute, 2007), and means were separated using the Ryan–Einot–Gabriel–Welsch multiple range test.

LD_{50} values for larvicidal data were calculated using SAS Proc Probit.[19] Control mortality was corrected using Abbott's formula.

3 RESULTS AND DISCUSSION

In the present research to synthesize biologically active hydrazide-hydrazones and 1,3,4-oxadiazoles, a total of 20 compounds were synthesized (Table 1). The purity of the synthesized compounds was checked by reversed-phase HPLC and elemental analysis. The structures of the compounds were supported by spectral data obtained by UV, IR, ^{1}H NMR and mass spectroscopy, which were in agreement with the proposed structures.[10,11] These hydrazide-hydrazone derivatives **1** to **10** and 3-acetyl-2,5-disubstituted-2,3-dihydro-1,3,4-oxadiazoles **11** to **20** were evaluated for repellent, biting deterrent and larvicidal activity against *Ae. aegypti*. The *in vitro* K&D system provided quantification of the mosquito feeding deterrent properties of hydrazone derivatives **1** to **20** (Fig. 1). The synthesized compounds were evaluated against 25 nmol cm^{-2} DEET as the positive control and acetone as the solvent (negative) control. The proportion not biting ranged between 0.8 and 0.92 in DEET and between 0.29 and 0.39 in the solvent control. Using this *in vitro* bioassay as a means to provide leads without needing humans to receive bites, it was apparent that the tendency of mosquito biting is related to the structure of the hydrazide-hydrazone derivatives. For better comparison of the compounds evaluated in the bioassays, the data were normalized by calculating the BDI. Based mean BDI values of these compounds are provided in Fig. 1. The BDI values show that all the compounds deterred biting at a higher level than acetone, the solvent control, except for compound **16**, where the BDI was not significantly different from 0 and the activity was not greater than that of acetone. The BDI values of compounds **1** and **17** were not significantly different from **1**, and statistically, the activity was not significantly different from that of DEET. Compound **1** is a hydrazone derivative with a non-substituted phenyl ring, and compound **17** is 1,3,4-oxadiazole with a 4-dimethylaminophenyl ring at the 2-position of oxadiazole. Compounds **17**, **4** and **3** followed compounds **1** and **17** in biting deterrent activity, with DBI values of 0.661, 0.511 and 0.45 respectively. When the structures of the active compounds were examined, hydrazones with 4-hydroxyphenyl (compound **4**), a 5-bromothiophene ring (compound **3**) and 1,3,4-oxadiazole with a furan ring (compound **17**) were more effective than the other derivatives. Compounds **1** and **17** were selected for cloth patch assay testing to determine the repellency level. This test employs human volunteers and was used to estimate the threshold concentration for repellency. A 537 nmol cm^{-2} concentration of compound **1** on cloth prevented bites through the cloth when worn on the arms of two of the volunteers, but was not repellent for a third volunteer at the highest concentration tested (2155 nmol cm^{-2}). For all three volunteers, compound **17** did not repel mosquitoes from biting at the highest concentration tested (1685 nmol cm^{-2}).

Hydrazide-hydrazone derivatives **1** to **10** and 3-acetyl-2,5-disubstituted-2,3-dihydro-1,3,4-oxadiazoles **11** to **20** were screened for their larvicidal activity at dosages of 100, 50 and 25 ppm against *Ae. aegypti* in high-throughput larval screening bioassays. Out of 20 compounds, compounds **10**, **11**, **12** and **17** showed 100% mortality and compounds **13**, **14**, **19** and **1** showed 50, 60, 20 and 10% mortality, respectively, at the maximum dose of 100 ppm, while the other compounds did not show any larvicidal activity (Table 2). LD_{50} and LD_{90} values of **10**, **11**, **12** and **17** are given in Fig. 2. Compounds **11** and **12** showed higher mortality, with LD_{50} values of 24.1 and 30.9 ppm respectively, than compounds **10** and **17** with LD_{50} values of 80.3 and 58.7 ppm at 24 h post-treatment. Similarly higher LD_{90} values in **11** and **12** (47.3 and 73.7 ppm) indicated the same trend of higher efficacy than **10** (207.9 ppm) and **17** (129.5 ppm). A similar pattern was observed at 48 and 72 days post-treatment. Oxadiazole derivatives carrying a phenyl, 4-bromophenyl and 4-dimethylaminophenyl ring displayed higher mortality than hydrazone derivatives, except for compound **10** with a furan ring. Compound **11**, with bromine on the phenyl ring, is more active compound than the non-substituted phenyl derivative (**10**), which can be explained by the high lipophilic feature.

Table 2. Mortality percentage in larvicidal screening bioassays of compounds **1** to **20** against first (24 h old) larvae of *Aedes aegypti*

Compounds[a]	Concentration (ppm)[b] 100	50	25
1	10	0	0
2	0	0	0
3	0	0	0
4	0	0	0
5	0	0	0
6	0	0	0
7	0	0	0
8	0	0	0
9	0	0	0
10	100	0	0
11	100	60	40
12	100	100	50
13	50	10	0
14	60	0	0
15	0	0	0
16	0	0	0
17	100	0	0
18	0	0	0
19	20	0	0
20	0	0	0

[a] Details of compounds 1 to 20 are given in Table 1.
[b] In screening data, each dose was repeated 2 times.

4 CONCLUSIONS

In this study, hydrazide-hydrazone derivatives **1** to **10** and 3-acetyl-2,5-disubstituted-2,3-dihydro-1,3,4-oxadiazoles **11** to **20** were evaluated for their biting deterrence and larvicidal activity against *Ae. aegypti* for the first time. Compound **17** showed the highest biting deterrent activity, and the deterrence was not significantly different from that of DEET in *in vitro* bioassays. Compound **1** also showed similar activity to DEET; however, these compounds did not show repellent activity in *in vivo* cloth patch bioassays against *Ae. aegypti*. These differences in activity in the two

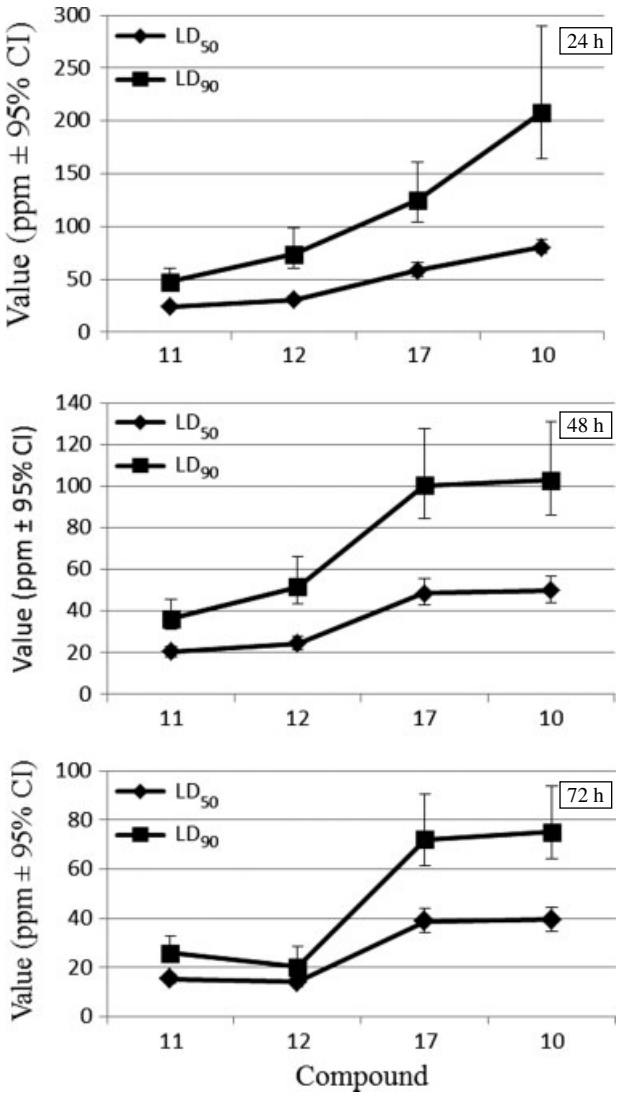


Figure 2. LD_{50} and LD_{90} values (± 95% CI) of **11**, **12**, **17** and **10** against one-day-old *Ae. aegypti* at 24, 48 and 72 h post-treatment.

bioassays are not entirely unexpected, as module-based feeding deterrency assays are designed to be rapid screens for activity and may at times overestimate repellency.[20]

Compounds **1** and **17** should not be considered for use as repellent at this stage because of insufficient activity in *in vivo* bioassays. The two 3-acetyl-2,5-disubstituted-2,3-dihydro-1,3,4-oxadiazoles, types **11** and **12**, showed the best larvicidal results. The structures of these two compounds should be modified further in order to explore the possibility of increasing larvicidal activity. This study appears to be the first report on such activity of both hydrazide-hydrazones (**1** to **10**) and oxadiazole derivatives (**11** to **20**).

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